

a.) Amendments to the Claims

1. (Currently Amended) A method for inducing differentiation of an embryonic stem cell into an ectodermal cell in vitro, which comprises culturing the embryonic stem cell under non-aggregation conditions, wherein said culturing is carried out in the absence of retinoic acid, and in the presence of a stroma cell or a stroma cell-derived factor.

2. (Previously Presented) The method according to claim 1, wherein the ectodermal cell is a cell which differentiates into a nervous system cell or an epidermal system cell.

Claims 3-11 (Cancelled).

12. (Currently Amended) ~~The A method according to claim 1~~ for inducing differentiation of an embryonic stem cell into an epidermal cell in vitro, which comprises culturing the embryonic stem cell under non-aggregation conditions, wherein said culturing is carried out in the absence of retinoic acid and in the presence of a stroma cell or a stroma cell-derived factor and bone morphogenetic protein 4.

13. (Currently Amended) ~~The A method according to claim 1~~, for inducing differentiation of an embryonic stem cell into a neural crest derived cell in vitro, which comprises:

culturing the embryonic stem cell under non-aggregation conditions,
wherein said culturing is carried out in the absence of retinoic acid, bone morphogenetic

protein 4 and sonic hedgehog, and in the presence of ~~sonic hedgehog~~ a stroma cell or a stroma cell-derived factor, and then

culturing the embryonic stem cell under non-aggregation conditions, wherein said culturing is carried out in the absence of retinoic acid, and in the presence of a stroma cell or a stroma cell-derived factor and bone morphogenetic protein 4.

14. (Currently Amended) The method according to ~~claim 1~~ any one of claims 1, 12, 13 and 73, wherein the non-aggregation conditions are conditions not mediating an embryoid body.

15. (Currently Amended) The method according to ~~claim 1~~ any one of claims 1, 12, 13 and 73, which further comprises culturing under serum-free culture conditions.

Claims 16-17 (Cancelled)

18. (Currently Amended) The method according to ~~claim 1~~ any one of claims 1, 12, 13 and 73, wherein the stroma cell is a stroma cell whose proliferation potency is deleted by a physicochemical treatment.

19. (Currently Amended) The method according to ~~claim 18~~ any one of claims 1, 12, 13 and 73, wherein the physicochemical treatment is selected from the group consisting of an antitumor agent irradiation and pathologic tissue fixative.

20. (Currently Amended) The method according to ~~claim 19~~ any one of

claims 1, 12, 13 and 73, wherein the physiocochemical treatment is an antitumor agent selected from the group consisting of mitomycin C, 5-fluorouracil, adriamycin and methotrexate.

21. (Currently Amended) The method according to ~~claim 19~~ any one of claims 1, 12, 13 and 73, wherein the physiocochemical treatment is selected from the group consisting of a microwave fixation, a rapid freeze-substitution fixation, a glutaraldehyde fixation, a p-formaldehyde fixation, a formalin fixation, an acetone fixation, a Van fixation, a periodic acid fixation, a methanol fixation and an osmic acid fixation.

22. (Previously Presented) The method according to claim 1, wherein the stroma cell is recognized by a monoclonal antibody produced by hybridoma FERM BP-7573.

23. (Currently Amended) The method according to ~~claim 1~~ any one of claims 1, 12, 13 and 73, wherein the stroma cell is selected from the group consisting of: a fetal primary culture fibroblast; an SIHM mouse-derived STO cell; a mouse fetus-derived NIH/3T3 cell; an M-CSF deficient mouse calvaria-derived OP9 cell; a mouse calvaria-derived MC3T3-G2/PA6 cell; an embryonic stem cell-derived stroma cell; and a bone marrow mesenchymal stem cell-derived stroma cell.

24. (Currently Amended) The method according to ~~claim 1~~ any one of claims 1, 12, 13 and 73, wherein the embryonic stem cell is selected from the group consisting of:

- (a) an embryonic stem cell established by culturing an early

embryo before implantation;

(b) an embryonic stem cell established by culturing an early embryo produced by nuclear transplantation of the nucleus of a somatic cell; and

(c) an embryonic stem cell in which a gene on the chromosome of the embryonic stem cell of (a) or (b) is modified using gene engineering.

25. (Canceled)

26. (Currently Amended) The method according to ~~claim 1~~ any one of claims 1, 12, 13 and 73, wherein the embryonic stem cell differentiates into an ectodermal cell or an ectoderm-derived cell at an efficiency of 5% or more.

27. (Currently Amended) The method according to ~~claim 1~~ any one of claims 1, 12, 13 and 73, which does not substantially accompany differentiation induction of a mesodermal system cell.

Claims 28-55 (Cancelled)

56. (Currently Amended) A method for evaluating a substance for activity in regulating differentiation of an embryonic stem cell into an ectodermal cell or an ectoderm-derived cell, which comprises:

carrying out the method according to any one of ~~claims 1, 2, 12-15, 18-24, 26, 27 or 72~~ claims 1, 2, 12, 13 or 22 both (i) in the presence of a substance to be tested and (ii) in the absence of the substance to be tested; and

comparing differentiation of the embryonic stem cell into an ectodermal cell obtained in the presence of the substance to be tested with that in the absence of the substance to be tested.

57. (Currently Amended) A method for screening a substance for activity in regulating differentiation of an embryonic stem cell into an ectodermal cell, which comprises:

carrying out the method according to any one of ~~claims~~ claims 1, 2, ~~12-15, 18-24, 26, 27 or 72~~ 12, 13 or 22 both (i) in the presence of a substance to be tested and (ii) in the absence of the substance to be tested; and

comparing differentiation of the embryonic stem cell into an ectodermal cell obtained in the presence of a substance to be tested with that in the absence of the substance to be tested.

Claims 58-71 (Cancelled).

72. (Currently Amended) The method according to ~~claim 1~~ any one of claims 1, 12, 13 and 73, wherein the ~~stem~~ stroma cell is PA6, OP9 or NIH3T3.

73. (New) A method for inducing differentiation of an embryonic stem cell into a neural tube derived cell *in vitro*, which comprises:

culturing the embryonic stem cell under non-aggregation conditions, wherein said culturing is carried out in the absence of retinoic acid, bone morphogenetic protein 4 and sonic hedgehog, and in the presence of stroma cell or a stroma cell-derived factor, and then

culturing the embryonic stem cell under non-aggregation conditions, wherein said culturing is carried out in the absence of retinoic acid, and in the presence of a stroma cell or a stroma cell-derived factor and sonic hedgehog.